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## Claims

- 5 1. A method of determining voltage changes by means of a voltage-sensitive dye, characterized in that the voltage-sensitive dye is irradiated with light having a wavelength at which the dye has an absorption ≤ 20% of its absorption maximum and the fluorescence caused by irradiation with light is measured.
  - 2. The method according to claim 1, characterized in that the waveleng th of the irradiated light is such that the dye has an absorption of ≤ 12%, in particular ≤ 8% and preferably ≤ 2% of its absorption maximum at said wavelength.
  - 3. The method according to claim 1, characterized in that the wavelength of the irradiated light is in the longer wavelength range, related to the absorption maximum.
- 4. The method according to any of the preceding claims, characterized in that an increase or decrease of fluorescence is measured.
  - 5. The method according to any of the preceding claims, characterized in that it is used to determine voltage changes in cells.
  - 6. The method according to any of the preceding claims, characterized in that it is used to determine voltage changes in membranes, especially cell membranes.
- 7. The method according to any of the preceding claims, characterized in that, as voltage-sensitive dye, a compound of formula (I)

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or formula (II)

is used, wherein

each R independently is a hydrocarbon residue, which optionally can be substituted with hydroxyl.

R1 is a monovalent residue,

n is an integer from 1 to 9, and

n is an integer from 0 to 8,

which compounds optionally can have one or more substituents at ring carbon atoms.

- 8. The method according to any of the preceding claims, characterized in that ANNINE-4, ANNINE-5, ANNINE-6, ANNINE-7, ANNINE-8 or ANNINE-9 is used as a voltage-sensitive dye.
- The method according to any of the preceding claims, characterized in that a change of fluorescence radiation caused by the Stark effect is measured.
- 10. The method according to any of the preceding claims, characterized in that a two-photon excitation is effected.
- 30 11. Voltage-sensitive dye having the formula (I)

or formula (II)

wherein

each R independently is a hydrocarbon residue, which optionally can be substituted with hydroxyl,

R<sup>1</sup> is a monovalent residue, n is an integer from 1 to 9, and n is an integer from 0 to 8,

which compounds optionally can have one or more substituen ts at ring carbon atoms.

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